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Research was conducted according to the principles enunciated in the "Guide for Laboratory Animal Facilities and Care," prepared by the National Academy cf Sciences - National Research Council.

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ABSTRACT

The specific resistivity of the internal medium of a variety of large cells was measured by a technique utilizing a single metal microelectrode subjected to alternating current in a circuit in which the voltage output varies with the conductivity of the thin layer of fluid at the electrode tip. The specific resistivity of cytoplasm of nerve cell bodies of *Anisodoris*, *Navanax* and *Aplysia* ranged from 434-1250 ohm cm whereas that of squid giant axon was 32-39 ohm cm. *Myxicola* giant axons had an average resistivity of 68 ohm cm whereas that for barnacle giant muscle fibers was 178 ohm cm and from *Amphiuma* red blood cells was 350 ohm cm. The temperature dependence of conductivities of *Aplysia* neurons and squid axon was not different from that of equally conductive salt solutions. The cause of the extraordinarily high resistivities of some of these tissues is unknown, but the results suggest some mechanism whereby interactions of ions with cell water and/or macromolecules lower ionic mobility.

I. INTRODUCTION

Biologic tissues consist primarily of inorganic salts, small organics, macromolecules and water. Cellular macromolecules may be structured in membranes but the other components are assumed for the most part to be dissolved in cell water. Both organic and inorganic molecules may have a layer of water tightly bound to charged and polar groups, called the water of hydration.[11,12] In most cases the water of hydration consists of one or several monolayers held by H^+-bond formation and with successive layers less tightly held. The remainder of cell water is usually considered to be unstructured. Although a sizeable fraction of some intracellular inorganic ions, such as Ca^{++} and Na^+, are bound to macromolecules,[30] most ions are presumed to exist in free solution.[18]

A simple test of these multiple assumptions can be made by a measurement of the electrical conductivity of the internal medium of cells. If the total concentration of the small ions in the cell is known, one can compare intracellular conductivity with that of a solution of comparable salt composition without cellular macromolecules. If there is a considerable difference, it is necessary to reconsider the assumption that ions, water and macromolecules coexist in the cell with only relatively minor interactions.

One of the earliest attempts to measure the electrical conductivity (or the inverse, resistivity) of the internal medium of cells was made by Höber.[19,20] Höber's technique consisted of applying high frequency alternating current across packed tissues. He studied primarily red blood cells since they were a uniform cell type and could easily be concentrated. Höber calibrated his apparatus by measurements of

solutions of known conductivity. He reported that the internal conductivity of red blood cells was equivalent to that of 0.1 percent NaCl. This value is considerably less than the conductivity of the external medium which is equivalent to 0.9 percent NaCl.

Measurements of intracellular conductivity of packed tissues using high frequency alternating currents (which bypass the membrane resistance) have been made on several tissues since the work of Höber. In 1926 Fricke and Morse[14] studied packed red blood cells at 800 Hz to 4.5 MHz and calculated an internal conductivity equivalent to 0.17 percent NaCl (a resistivity 5 times that of the external medium). In 1928 Cole[7] studied Arbacia (sea urchin) eggs and measured a specific resistance of the interior of the egg of about 90 ohm cm (3.6 times that of the external medium). Thompson[35] measured conductivity of packed frog muscle and determined that the internal medium was as conductive as 0.13 to 0.21 percent NaCl solutions (resistivity 4.3-6.9 times that of frog Ringer). A more recent study on frog muscle[26] using similar techniques reported conductivity equivalent to 0.3 percent NaCl. By soaking the muscle in sucrose this value could be reduced to be equivalent to 0.2 percent NaCl. If equilibrated in twice concentrated Ringer's solution, the conductivity rose to that of 0.65 percent NaCl.

Schwan and collaborators have studied many kinds of packed tissues at frequencies of 0.5 to 250 MHz. In a study of red blood cells of various species Pauly and Schwan[27] found dielectric constants to be similar in all species and internal resistivities averaged about 200 ohm cm. This value is 2.7 times greater than expected, and they attributed the high resistivity to hydrodynamic and electrostatic effects which

depress ionic mobility. Even on consideration of the volume concentration of hemoglobin and its water of hydration the resistivity was twice that expected. Schwan and Li[34] studied a number of packed tissues (skeletal and heart muscle, liver, kidney, lung and fat). The conductivity of most of these tissues was less than that of 0.9 percent NaCl by a factor of 2-3, while that of fat was less by a factor of 10. These results were obtained, of course, from studies on tissues which are not homogeneous and where there is a sizeable extracellular space.

For a variety of reasons these conductivity measurements have received relatively little attention. There is no simple explanation for the observation that internal conductivity of many tissues is lower than expected. Furthermore, the interpretation of data from packed tissue is more difficult than observations made on single cells.

Various attempts have been made to measure cytoplasmic conductivity of single cells, beginning with Hartree and Hill[16] who studied frog muscle fibers. They found internal conductivity was only one-half that of the NaCl solution with which the muscle was in osmotic equilibrium and attributed the low conductivity to hindrance by membranes. Gelfan[15] measured intracellular conductivity of giant algae and protozoa by passing current pulses between two intracellular microelectrodes separated by a fixed distance. He found internal conductivity in these cells considerably higher than that of the external medium (dilute fresh water). Schanne[32] has recently described another technique for single cell conductivity measurement, based on the fact that the resistance of an electrode changes with solution resistivity. This technique has been applied to crayfish muscle fibers by Law and Atwood,[24] who measured internal resistivities of about 150 ohm cm. However, serious criticisms of this technique

have been made by Peskoff and Eisenberg[28] who have shown that a considerable portion of the voltage which is thought to reflect resistivity is dependent upon electrode position.

The most unambiguous measurements of protoplasmic conductivity have been made in the squid giant axon. This axon is of sufficient size so that conductivity can be measured in the axon or in extruded axoplasm. Cole and Hodgkin[9] and Cole and Moore[10] reported axoplasmic resistance to be between 1.2 and 1.4 times that of sea-water. There is some indication of variability from preparation to preparation, since Cole[8] reports experiments on two axons showing conductivity near to that of the external medium. Because of the direct measurements and the easily interpreted results from squid axons most workers have generalized these findings and concluded that ions in cells can carry current with nearly as much ease as in free solution.

We have recently presented a theoretical and procedural basis for use of a new technique to measure the internal conductivity of cells.[6] The procedure is a modification of that described by Bak[1] for testing metal microelectrodes. Alternating current, usually at 100 kHz, is passed between the tip of the microelectrode and an extracellular reference. The voltage output reflects the ability of the thin layer of solution at the tip of the microelectrode to carry current at the applied frequency. The output is sensitive to the polarization impedance of the electrode, frequency, solution conductivity and the electrode dimension. When each electrode is individually calibrated in solutions of known conductivity it can be used to measure the local conductivity in cells. Our previous studies have been primarily on Aplysia neurons, where internal conductivity was found to be only 5 percent that of the external solution.[6]

The present study extends conductivity measurements to other large single cells, and demonstrates that there is a wide variability of internal conductivity among tissues.

II. METHODS

Conductivity was measured with glass insulated gold or platinum microelectrodes as previously described.[5,6] Electrodes were individually calibrated in dilutions of seawater or frog Ringer's solution of known conductivity. All measurements were made at 100 kHz except in experiments where frequency was varied. In such experiments a Krohn-Hite model 4200 oscillator was used and frequencies were varied from 25 to 300 kHz. Plots of output voltage as a function of frequency were obtained using a Tektronix 502 oscilloscope with frequency displayed on the X axis and voltage on the Y axis. Records were photographed on Polaroid film from the oscilloscope face.

All experiments were performed at room temperature except those in which temperature was varied and is specified. Cooling was achieved by flowing the perfusing solution through coiled tubing in ice.

Experiments on the ganglia of squid, _Aplysia_, _Anisodoris_ and _Navanax_ were made by removing the tissue from the animal, pinning it to a layer of Sylgard (Dow Corning) in a Lucite chamber and maintaining the preparation under flowing artificial seawater. The connective tissue capsule over the nerve cells was slit with a sliver of a razor blade in most experiments. A separate chamber was used for experiments on axons and barnacle muscle fibers. This chamber allowed the axon to lie across a pair of Ag-AgCl electrodes which could be used for stimulation.

Amphiuma red blood cells and frog oocytes were studied in a dish with a layer of 3 percent agar-agar at the bottom. The eggs and red blood cells would settle on the

agar and this allowed penetration without damage to the microelectrode. Oocytes were dissected from gravid female frogs and were unfertilized.

For intracellular recording of conductivity the metal microelectrode was inserted into the cell under direct visualization. For the nerve and muscle cells simultaneous recordings were made with a glass micropipette filled with 3 M KCl to measure membrane and action potentials. Cells were not studied if they had electrical parameters indicating injury (action potentials less than 80 mV, resting potentials less than 40 mV). In excitable cells action potentials were recorded through the glass pipette (DC coupled) and through the metal electrode (AC coupled) to insure that both electrodes were in the same cell. Conductivities were determined only with electrodes on which complete calibration curves were obtained and where the electrode showed identical readings before and after penetration.

The voltage output of the experimental apparatus is given by equation (1) (see Discussion). The output varies with source voltage and feedback resistance as well as electrode impedance which is, in turn, a function of frequency and several electrode properties in addition to local conductivity. Under the experimental conditions all of these factors but local conductivity are kept constant. In our previous publications,[5,6] voltage output was defined as "equivalent capacitance" and numerical values were obtained by calibrating with known capacitors. This term is, unfortunately, without meaning, and thus in this and future publications the output voltage will be plotted directly. The specific resistivity of tissues was obtained by determining the dilution of the external medium with the same voltage output measured in the cell, and measuring the resistivity of that solution in a conductivity cell.

III. RESULTS

<u>Characteristics of the electrode as a function of frequency</u>. Figure 1 illustrates an attempt to describe the properties of the electrodes in terms of their resistive and capacitive components. Part A is a record of the voltage output as a function of

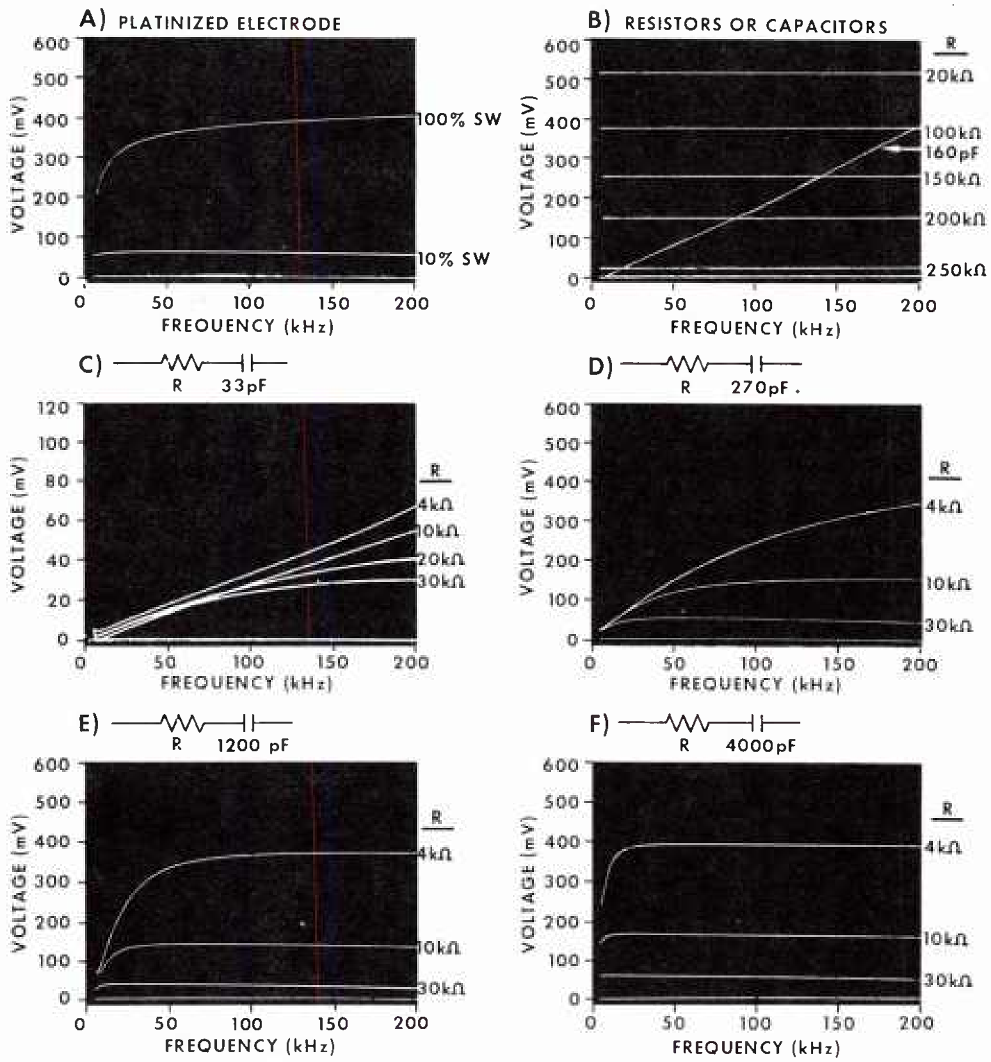

Figure 1. Voltage output of the recording circuit as a function of frequency (A) for 1 mil gold wire embedded in glass, polished and platinized in solutions of 10 and 100 percent seawater; (B) for resistors and capacitors; (C-F) for resistors and capacitors in series

frequency in seawater and 1:10 dilution from an electrode made of 1 mil gold wire which was embedded in glass and polished on end. The uninsulated polished surface was platinized. In general, platinized gold wire behaves in a similar fashion to the platinum microelectrodes used in most experiments but has the advantage that it has a greater surface area. When frequency is swept from 5 to 200 kHz in normal seawater, the voltage output increases rapidly and approaches a plateau. When the electrode is tested in a 10 percent seawater:90 percent distilled water solution, the voltage output is reasonably flat over the whole frequency range. Part B illustrates the voltage output obtained from resistors and capacitors. The output from resistors decreases as the magnitude of the resistor increases and does not vary with frequency. The voltage output from capacitors, however, increases linearly with increasing frequency. These results are as expected from the equations describing the voltage output of this assembly.[6] Parts C through F show voltage output as a function of frequency for various combinations of resistors and capacitors in series. For a given capacitance the voltage output decreases with increasing resistance. When capacitance is small, as in C, the voltage output is primarily influenced by the capacitance and at low resistances increases linearly with frequency. As capacitance is increased the voltage output becomes more and more similar to that for a pure resistance except at very low frequencies. In F, at 4000 pF, the output is flat at all but low frequencies. The output from the gold wire in 100 percent seawater is similar to that from 4000 ohms in series with 1200 pF.

Conductivity measurements in cells require use of microelectrodes which have a smaller surface area than the 1 mil wire used in Figure 1. Figure 2 illustrates the

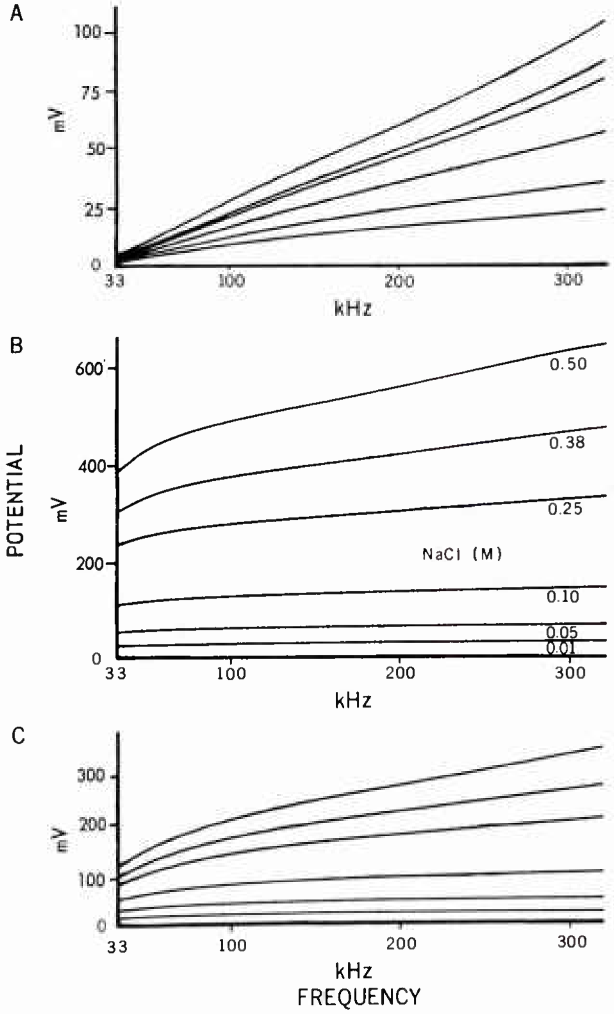

Figure 2.
Conductivity as a function of frequency measured with a typical glass-insulated microelectrode (A) before deposition of platinum black; (B) after platinization and (C) after multiple penetrations of muscle fibers, causing partial loss of the coat of platinum black

voltage output measured by the same electrode at three different stages of its use in various concentrations of NaCl solutions. Part A shows conductivity as a function of frequency recorded by the bare platinum microelectrode before it was coated with platinum black. Under these conditions the electrode behaves like a pure capacitor, and output increases with frequency. The voltage output is very much increased by the deposition of platinum black, presumably as a result of the increase in surface area at the tip of the electrode (B). Furthermore, now at low concentrations of

sodium chloride the electrode functions as though it were a resistance, not a capacitance. This is primarily a result of the fact that platinization greatly reduces the polarization impedance[33] which is frequency dependent. At higher concentrations there is a slow increase in voltage output with frequency. Part C shows the response from the same electrode after it had been used for numerous penetrations of a barnacle muscle fiber. The overall response as a function of frequency is similar to that of the newly platinized electrode, but the voltage output is less. This usually occurred on repeated use of an electrode and presumably reflects the loss of platinum black on repeated penetration of the cell membrane. Because the coat of platinum black is fragile, we have taken pains to always calibrate the electrodes before and after measurements inside of the cell, and to consider only those penetrations where the calibration did not change significantly with penetration.

Conductivity measurements in cells. Table I shows results of measurements of conductivity in the giant nerve cell bodies of three molluscs, the opisthobranchs *Aplysia californica* and *Navanax inermis*, and the nudibranch *Anisodoris nobolis*. In

Table I. Conductivity of Nerve Cell Bodies

Preparation	Number	Equivalent isotonic salt concentration (percent)	Specific resistivity (ohm cm)
Seawater		100	25
Aplysia	42	6 (4-7)	434
Navanax	15	4 (2-8)	625
Anisodoris	20	2 (1-3)	1250

agreement with our previous observations on Aplysia,[6,21] these neurons have an internal conductivity corresponding to that of a solution of 6 percent seawater in distilled water or an internal resistivity of 434 ohm cm. The external medium (seawater) has a specific resistance of 25 ohm cm. The specific resistivity in Navanax neurons averaged 625 ohm cm; whereas, for Anisodoris, resistivity was 1250 ohm cm.

Figure 3 shows an experiment on a neuron from the visceral ganglion of Anisodoris with voltage recordings from the glass pipette (A-1, B-1) and conductivity (A-2) and voltage (B-2) recordings from the metal electrode. The conductivity recorded in this neuron is equivalent to that of 3 percent seawater.

The low internal conductivity in these neurons appears quite uniform through the interior of the cell. No differences have been observed on a systematic examination of different areas of the neuron with a small electrode, even though it is likely that at some time the electrode is actually in the lobulated nucleus. The range of values obtained for each type of neuron shows relatively little variability, and this fact further supports the postulate that the conductivities measured are representative of the entire cell body.

The values obtained for internal resistivity of these molluscan nerve cell bodies are very different from those known for squid giant axon from the work of Cole and Hodgkin[9] and Cole and Moore.[10] In order to confirm their measurements and further test the use of this technique we have studied the giant axons of both California (Loligo opalescens) and Atlantic (Loligo pealei) squid. Results are presented in Table II and show that the resistivity of most stellar axons of both species (200-800 μm diameter) is between 30-40 ohm cm. These measurements show a considerable range

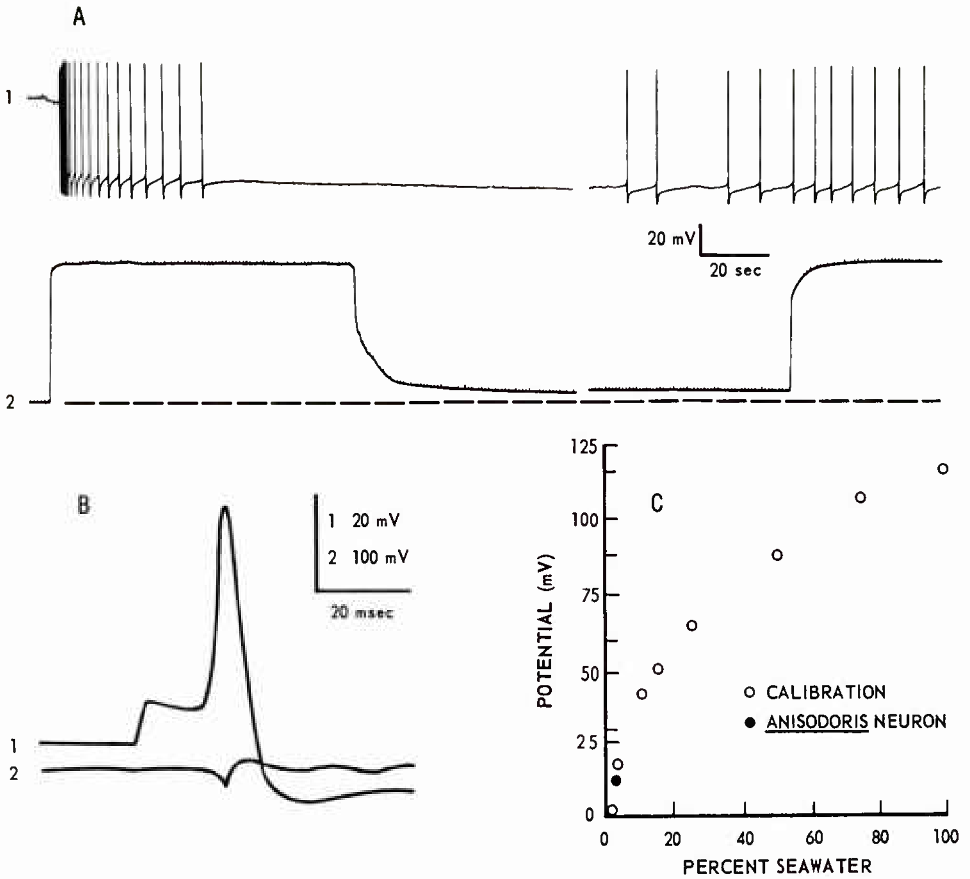

Figure 3. Conductivity and DC intracellular recordings from a neuron in the visceral ganglion of Anisodoris. Trace 1 in A and B are penwriter and oscilloscope traces respectively of the intracellular potential and activity recorded through a glass pipette. Penetration of the neurons occurs at the extreme left of A-1. Trace 2 in A records conductivity. The electrode is initially in air, giving the zero reading which is thereafter indicated by the dashed line. As the electrode tip is lowered into the seawater, the output increases to read 110 mV (extreme left). At left center in A-2, the neuron is penetrated with the metal electrode, and when the uninsulated tip is totally in the cell, the conductivity falls to 12 mV. This value corresponds to that of 3 percent seawater, as indicated by the calibration curve of this electrode, shown in C. Part B shows that the metal conductivity electrode is in the same neuron as the glass pipette, since an action potential initiated by passage of depolarizing current through the glass pipette is recorded (AC coupled) through the metal electrode (B-2). The right portion of A shows the return of conductivity to control value when the metal electrode was withdrawn from the cell into seawater.

from one axon to another. One possible cause of variability is suggested by the relatively few observations on the variety of giant axons exiting from the isolated stellate ganglion. In these experiments the stellar axon, which is always the largest, tended to have a greater conductivity than did smaller giant axons.

Table II. Conductivity of Giant Axons

Preparation	Number	Equivalent isotonic salt concentration (percent)	Specific resistivity (ohm cm)
Seawater		100	25
Loligo opalescens	13	78 (61-88)	32
Loligo pealei	15	65 (49-105)	39
From ganglion			
Stellar axon	5	69 (55-82)	36
Smaller axons	6	55 (55-70)	45
Myxicola			
In normal seawater	6	37 (30-44)	68
In hypertonic seawater (125%)	13	47 (39-60)	53

The difference in internal conductivities of neuronal soma and squid axons might represent a fundamental difference between axons and cell bodies. To test this hypothesis we studied the giant axons of the marine annelid Myxicola infundibulum. Figure 4 shows such an experiment, performed on an axon perfused with a high Mg^{++} (150 mM) seawater. Although such a solution is 125 percent of normal osmolarity it is advantageous because the contraction of adherent muscle fibers on axonal discharge is blocked. Part A shows the conductivity recording. At the beginning of the trace the electrode is in seawater. When lowered against the axon, the reading transiently decreased

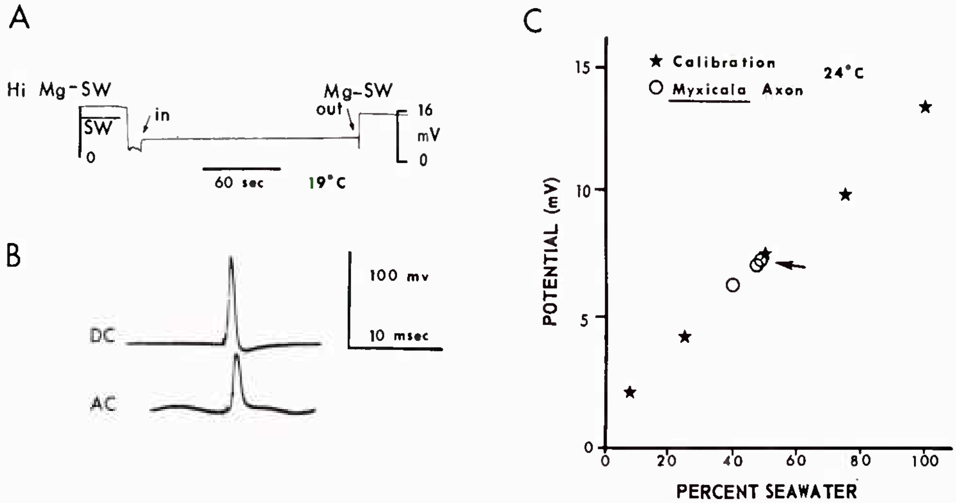

Figure 4. Conductivity measured in an isolated giant axon of Myxicola in seawater with 150 mM Mg^{++} to stop muscle contraction. Recording procedures are as in Figure 3. A shows conductivity recording, B shows action potential recorded through a glass pipette (DC) and the metal conductivity electrode (AC). C shows the electrode calibration curve, with open circles representing three Myxicola axons studied with this electrode. The arrow indicates results from the axon illustrated in A and B.

and was unstable as the electrode tip was forced against the axonal membrane. At the arrow marked "in" the electrode could be seen to penetrate the membrane and enter the axon. The conductivity recorded in the axon was equivalent to a 49 percent seawater solution. At the arrow marked "out" the electrode was withdrawn from the axon and the reading returned to control value. The action potential was about 90 mV (B-DC) and the simultaneous recording of an action potential through the AC electrode demonstrates that both electrodes were satisfactorily placed in the axon. Thirteen axons were studied in satisfactory detail in hypertonic high Mg^{++} seawater and these axons, as shown in Table II, had an average specific resistivity of 53 ohm cm. It is likely

that this value of specific resistivity is lower than that in fact found in Myxicola axons. In six axons studied in normal seawater the average resistivity was 68 ohm cm. Although this is a small sample due to the technical difficulty of keeping the electrode in a moving axon it seems likely that the hypertonic solution used to depress synaptic transmission caused a loss of cell water, leaving behind a more conductive medium. Nonetheless, this axoplasm is about 10 times as conductive as the internal medium of molluscan nerve cell bodies.

Since these results suggest some fundamental difference in the electrical properties of the axoplasm and somatoplasm we have examined the variation of conductivity as a function of frequency (Figure 5) from an Aplysia neuron (A) and a squid axon (C). B and D are the calibrations in various dilutions of seawater for the electrodes used in A and C respectively. In these experiments the conductivity in the Aplysia neuron corresponds to a dilution of 4 percent seawater whereas the conductivity of the squid axon corresponds to a conductivity of 50 percent seawater. In neither case does the shape of the conductivity versus frequency response differ in any significant regard from the response obtained in the solution of approximately the same conductivity.

Figure 6 shows the temperature dependence of conductivity of squid axoplasm and Aplysia somatoplasm as compared to that of dilutions of seawater with approximately comparable conductivities. Part A is from an experiment with an axon with a resistivity of 50 ohm cm (equal to 50 percent seawater). Part B shows a similar experiment on an Aplysia neuron which has a conductivity equivalent to 4 percent seawater. The variation of conductivity with temperature recorded in the cells does not differ significantly from that of the solution of similar conductivity. Thus there are

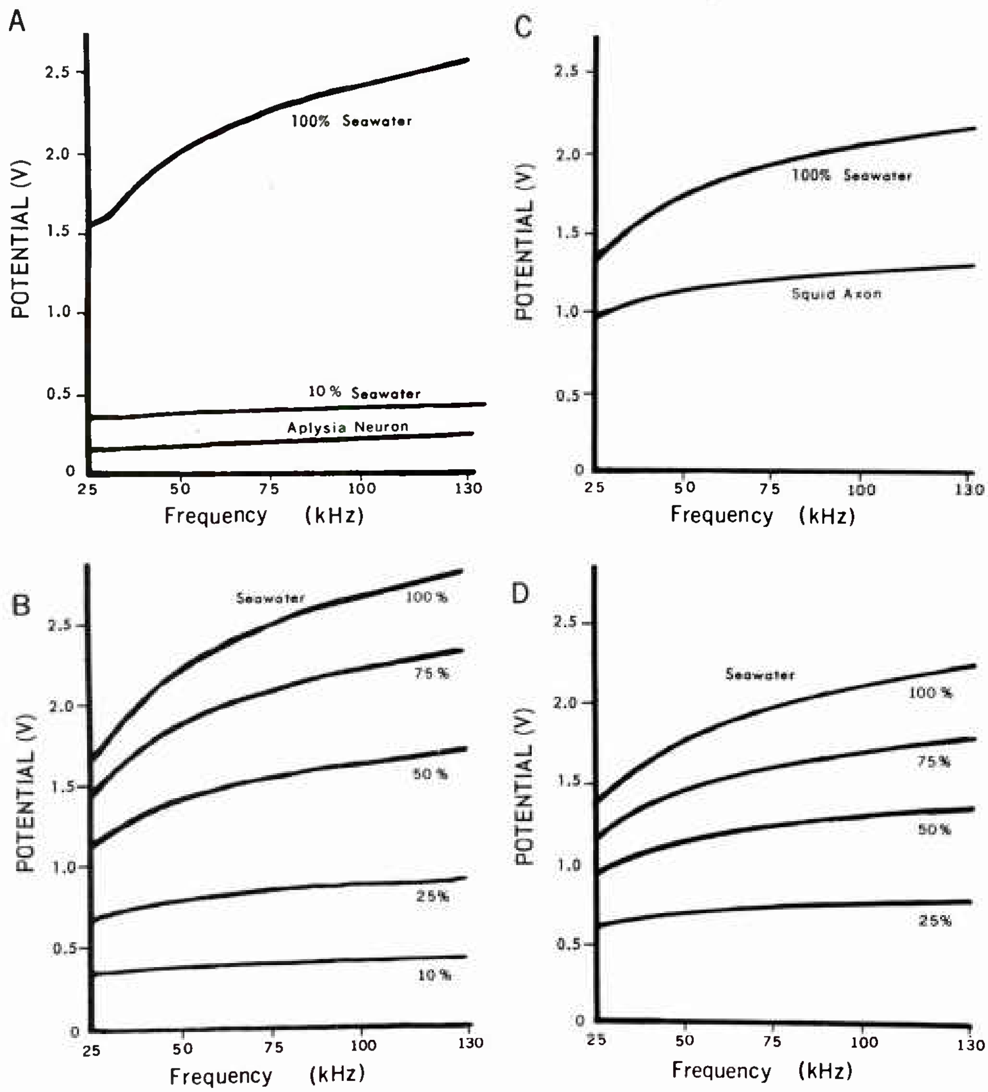

Figure 5. Voltage output as a function of frequency in an Aplysia neuron (A) and a squid axon (C). Parts B and D are calibrating traces in the indicated dilutions of seawater for the electrodes used in A and C respectively.

no obvious peculiarities of frequency or temperature dependence of the low conductivity in Aplysia neurons which might provide a clue to the basis for the differences with squid axon.

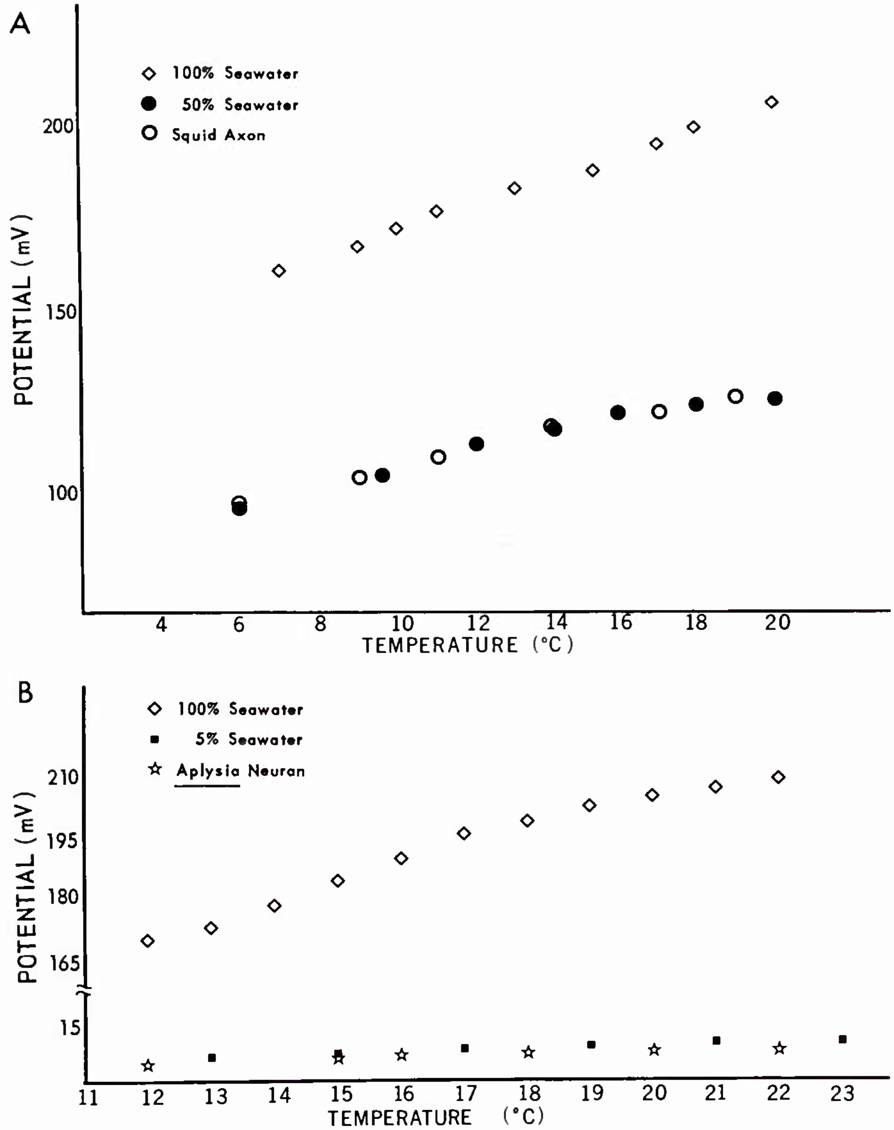

Figure 6. Temperature dependence of conductivity of squid axon (A) and an Aplysia neuron (B). In each case the temperature dependence of conductivity of 100 percent seawater and a dilution with a similar conductivity as the tissue is shown.

Figure 7 shows results from an experiment measuring conductivity of a giant muscle fiber from the barnacle Balanus nubilis. Part A demonstrates one penetration at a temperature of 14°C. The electrode was visually observed to penetrate the

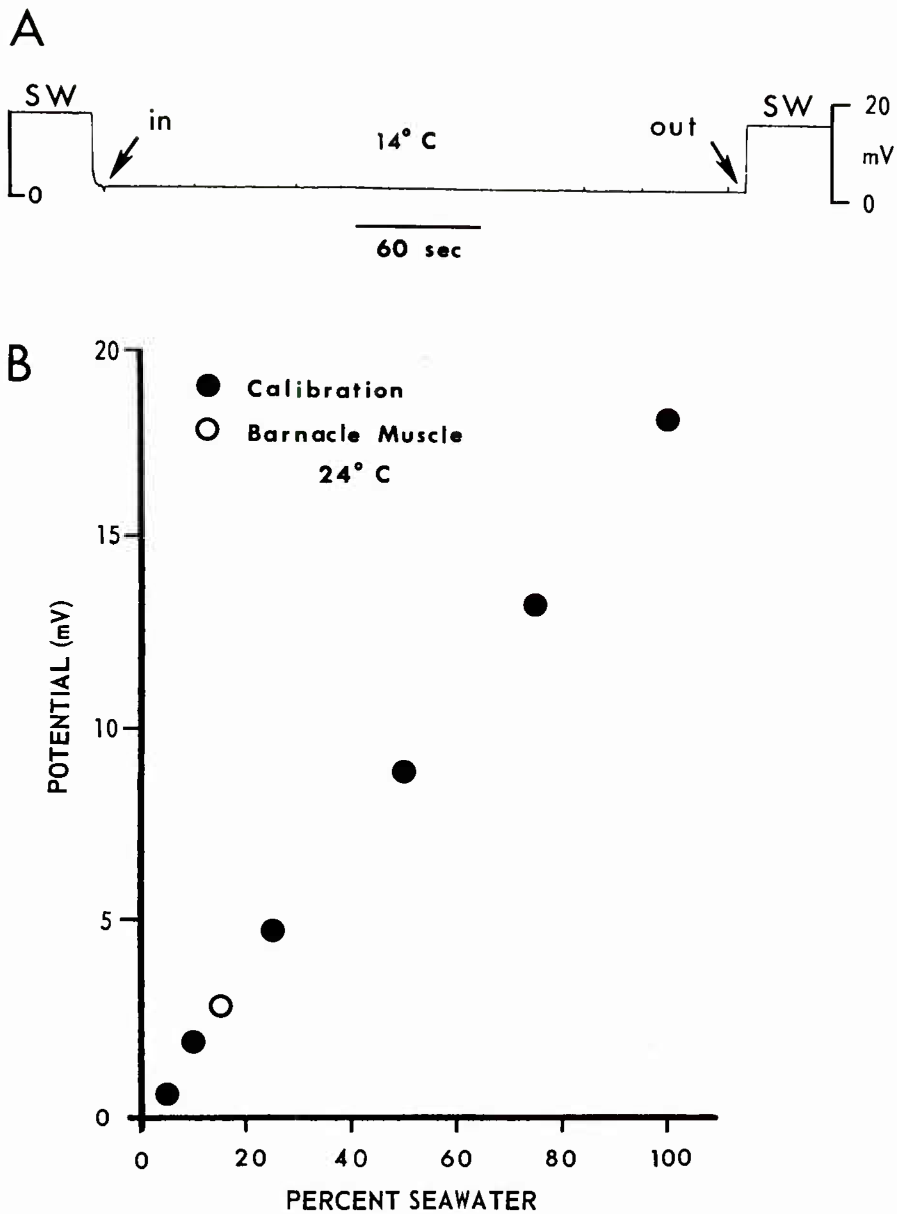

Figure 7. Conductivity measured in a barnacle muscle fiber (A) and the electrode calibration (B). Record A was taken at 14°C while B was at 24°C.

muscle fiber at the arrow marked "in" and was withdrawn at the arrow marked "out", and gave the same reading in the seawater solution as just before penetration. Part B shows the calibration of the electrode taken at room temperature. The open circle

indicates the value for conductivity taken in a different penetration of the same muscle fiber at room temperature. In this case the conductivity within the muscle fiber corresponds to that of the solution of 18 percent seawater. Table III shows results of conductivity measurements from 16 barnacle fibers in which the average equivalent isotonic salt concentration was a solution of 14 percent seawater, giving a calculated specific resistivity of 178 ohm cm.

Table III. Conductivity of Muscle, Egg and Blood Cells

Preparation	Number	Equivalent isotonic salt concentration (percent)	Specific resistivity (ohm cm)
Seawater		100	25
Barnacle muscle fibers	16	14 (10-23)	178
Frog Ringer		100	77
Frog oocytes	12	40 (30-50)	190
Amphiuma RBCs	47	22 (11-41)	350

Measurements of conductivity were also made in frog oocytes and *Amphiuma* red blood cells. These results are shown in Table III. The frog oocytes show a rather marked variability with the average specific resistivity equivalent to 190 ohm cm. Measurements of conductivity of 47 red blood cells indicate an equivalent isotonic salt concentration of 22 percent frog Ringer and a specific resistivity of 350 ohm cm.

IV. DISCUSSION

Possible errors resulting from use of this technique for conductivity measurement. The voltage output V_o for the recording system used in these experiments is:

$$V_o = \left| \frac{R_1}{Z_x} \right| V_s \tag{1}$$

where R_1 is a feedback resistance, V_s is the source voltage and $|Z_x|$ is the input impedance of the microelectrode in its local environment.[6] Since R_1 and V_s are held constant, V_o varies inversely with $|Z_x|$.

The input impedance of the electrode has the form

$$Z_x = \frac{\rho}{4\pi a} + \frac{\bar{\gamma}}{4\pi a^2 (j\omega)^{\alpha}} \tag{2}$$

where the first part represents the electrode impedance and the second the polarization impedance. (ρ is specific resistance of the medium in ohm cm, a is electrode area, γ is specific polarization impedance, ω is frequency and the exponent α is characteristic of a given electrode system and has a value of 1 for a pure capacitance, 0 for a pure resistance or values in between for combinations, and j is equal to the square root of minus one.) Use of this technique is valid if the only difference between calibrating solutions and tissue measurements is in ρ, and not R_1, V_s, α, γ, ω, or a. The most serious difficulty in keeping these factors constant is in maintaining the coating of platinum black. The application of the platinum black is important to increase surface area and particularly to reduce the polarization impedance.[33] It is fragile, however, and easily rubbed off with electrode use, necessitating repeated

calibration of electrodes and use of measurements only when calibration does not change.

The degree to which the polarization impedance influences V_o is the ratio of polarization to electrode impedance and is equal to

$$\Delta = \frac{\bar{\gamma}}{\rho \ a \ (j\omega)^{\alpha}} . \tag{3}$$

Thus, the polarization impedance will have little effect if γ is small, conductance $(\frac{1}{\rho})$ is small, ω is high and a is large. Although it is very much preferable to have polarization impedance small, the use of this technique to measure conductivity is still valid in the presence of appreciable polarization impedance, provided that it is not different in calibrating and testing measurements.

The output voltage of the large electrode shown in Figure 1 is very similar to that of a 4000-ohm resistor in series with a capacitor of 1200-4000 pF, where V_o increases at low frequencies to a value which is then unchanged with further increases in frequency. However, most microelectrodes showed to varying degrees a further slow increase in V_o at higher frequencies. It is likely that this results from a significant polarization impedance, since the electrode impedance is not frequency dependent. However, as shown in Figure 4 for two electrodes which show pronounced increases in V_o with frequency, the form of the V_o frequency relationship is identical in tissues of either high or low internal conductivities to that of an equally conductive calibrating solution. This observation justifies comparison made between intracellular measurements and calibrating solutions at one frequency.

It is possible that the low apparent conductivities in some tissues are artifacts of an adhesion of intracellular proteins or membranes to the electrode tip. As shown in Figure 4, when the electrode is lowered against the external membrane of a giant axon, there is an apparent unstable decrease in V_o until the electrode pops through the membrane. Intracellular macromolecules or membranes might cause a similar depression. Although this possibility cannot be eliminated, it appears unlikely for several reasons. The shift in V_o on penetration of cells was sudden and stable. The value was consistent from cell to cell on multiple penetration of the cell and at multiple sites within a single penetration even when the electrode was being withdrawn and should be pulling away from intracellular structures. We have previously shown that this technique records conductivity identical to that measured with a standard conductivity cell in solutions of salt and water with added high concentrations of sugars, carbohydrates and proteins.[6] Most importantly, the values of intracellular conductivity reported in the present study are in close agreement for the respective tissues to those obtained by other techniques for squid axon,[9,10] red blood cells[14,19,20,27] and Aplysia neurons.[21] In the latter study of Aplysia neurons conductivity was determined through use of a four-pole array of microelectrodes, with current pulses passed between the outer two electrodes, and the voltage deflection was measured between the center pair and compared to that obtained in calibrating solutions of known conductivity. Thus, the resistance to current flow across about 125 μm of tissue separating the center electrodes was determined, and was found to be identical to the value measured by use of the single electrode method. This observation indicates that not only do intracellular membranes not interfere with measurements of conductivity, but also suggests

that the resistance across such membranes is not much greater than that of the rest of the cytoplasm.

Another possible error in these measurements is a result of the asymmetric current distribution in the cell. This problem of the three-dimensional distribution of electric fields in cells has been recently examined by Eisenberg and Johnson,[13] Barcilon et al.[2] and Peskoff and Eisenberg.[28] They have used singular perturbation analysis to describe mathematically the distribution of current when applied intracellularly from a point source, and have shown that asymmetries of current distribution can lead to sizeable errors in interpretation of electrical measurements. Eisenberg and Peskoff have examined the method used in the present experiments as well as the use of the four-electrode array used by Hovey et al.[21] in a similar analysis and have calculated that the error resulting from asymmetries in current distribution in each of these kinds of measurements can at most be a factor of two (personal communication). Even if the error is a factor of two, it cannot explain the very low conductivities measured in the neuronal cell bodies and the dramatic difference between the axons and cell bodies.

<u>What is the cause of low intracellular conductivity</u>? Low intracellular conductivity might result from (1) low ionic concentrations, (2) binding of ions to macromolecules, (3) structuring of cell water, creating a microviscosity which retards ionic mobility, or (4) some combination of the above. However, the ionic content of nerve cell bodies is not very different from that of squid axon and, while some degree of ion binding is probably characteristic of most tissues, it is apparently not great enough to explain the deviation from the expected conductivity of molluscan nerve cell bodies

(see Carpenter et al.[6] for references). A very high microviscosity, presumably resulting from structuring of water, has been observed using a fluorescent probe in yeast cells,[3] and by use of electron spin resonance probes in yeast, bacteria, algae and other cultured human cells by Keith and Snipes.[23] However, other studies of water in many biological systems suggest that it is not different from that of free solution.[12]

All of the tissues studied in these experiments except the giant axons had internal conductivities lower than expected. At present, the reason that conductivities are so low is not clear. Whatever the cause, it does not have unusual temperature dependence (Figure 6). The most obvious difference between the tissues with high conductivities, such as squid axon, and those with low conductivity, such as barnacle muscle and nerve cell bodies, is the amount of protein and membranous organization.

The observation that internal resistivity of barnacle muscle fibers is 178 ohm cm is of particular interest. Barnacle fibers have been studied extensively by Hinke and co-workers[17,18] who report ionic concentration and activities measured with ion-specific microelectrodes. They conclude that, although a fraction of both fiber water and ions are bound, 68 percent of fiber water, 27 percent of Na^+, 87 percent of K^+ and 69 percent of Cl^- are free.

The microviscosity of single barnacle fibers has been measured by Sachs and Latorre[31] using the electron spin resonance of a nitroxide probe which distributes in cell water. They found the microviscosity in the fiber to be no more than fivefold and tenfold greater than that of free solutions. A microviscosity this great would explain the low conductivity of barnacle fibers.

With present information, it appears likely that a high microviscosity caused by some degree of structuring of cell water is the primary cause of low intracellular conductivity. The microviscosity is probably not uniform throughout the cell, but is greater in proximity to polar and charged groups of cellular macromolecules. Binding of ions to macromolecules certainly contributes to some extent as well,[18,30] but is not in barnacle adequate to explain the low conductivity. It appears more likely that ions are trapped in the partially structured water and consequently cannot carry current. In the extreme case the interior of a cell may resemble the situation proposed by Ling,[25] where all ions and water are structured. However, this is not the situation for all tissues, as indicated by the high conductivity of squid axoplasm. Our results suggest a wide range of organization of water and ions among biological tissues, dependent upon the amount and charges of cellular macromolecules. This conclusion is in basic agreement with the observations of Keith and Snipes.[23]

Functional significance of low intracellular conductivity. The results of this investigation confirm the observation that the conductivity of squid axoplasm is only slightly less than that of the external solution.[9,10] In addition, these results confirm the observations, made in several laboratories throughout this century on packed red blood cells, eggs, muscle and other tissues, that the internal milieu of the majority of biologic tissues is considerably less conductive than expected. In toto these results show that the frequent assumption that internal conductivities of all tissues are similar to the conductivity of squid axon is totally unjustified.

Although it has not yet proven possible to measure conductivity of a soma and axon from the same preparation, it appears that in general axons have a more

conductive internal medium than do cell bodies. This is to say that the resistive properties, and as a consequence the electrical properties, of a neuron are not the same throughout the cell. Furthermore, it is likely that specific membrane resistance also is not uniform over the neuron, since Carpenter[4] has shown that the nerve cell bodies which give rise to the squid giant axon have a specific membrane resistance nearly 30 times greater than that of the axon.

Most important electrical properties of cells appear to be functions of the outer cell membrane. However, the internal resistivity will influence some cellular functions in important ways. If internal resistivity is high and current is applied through an intracellular electrode, the assumption that all significant voltage drop occurs only at the external membrane is not valid. As a result the cell will not be isopotential and voltage space clamping cannot be accomplished. This is, in fact, the situation with giant *Aplysia* neurons.[22]

Another important parameter, especially in the vertebrate nervous system, is the length constant λ which has the form

$$\lambda = \left(\frac{R_m}{R_e + R_i}\right)^{1/2} \tag{4}$$

where R_m is specific membrane resistance and R_e and R_i are the specific resistance of external and internal media respectively. If R_i is increased, λ is shortened. It is possible that in most biological systems with a high R_i, R_m is also high, such that the length constant remains relatively constant. However, the present study strongly indicates that R_i cannot be considered very low without a direct measurement.

The values of R_m and R_i in dendrites of mammalian neurons have special importance in determining what, if any, effect synaptic input on distal dendrites has on neuronal excitability. There are at present no direct measures of either R_m or R_i in dendrites. Rall[29] has presented an elegant mathematical model of the neuron which analyzes the effect of synaptic inputs on dendrites at various distances from the spike generating region. If one assumes that there is uniformity of R_i and R_m throughout the neuron and that an average value of R_i is 50 ohm cm, this model suggests that even distal dendritic inputs have effects on soma excitability. This conclusion would obviously have to be reexamined if there is not membrane uniformity and if R_i is much larger than 50 ohm cm. These considerations suggest that it is important to achieve direct measurements of R_i and R_m in dendrites.

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13. ABSTRACT

The specific resistivity of the internal medium of a variety of large cells was measured by a technique utilizing a single metal microelectrode subjected to alternating current in a circuit in which the voltage output varies with the conductivity of the thin layer of fluid at the electrode tip. The specific resistivity of cytoplasm of nerve cell bodies of Anisodoris, Navanax and Aplysia ranged from 434-1250 ohm cm whereas that of squid giant axon was 32-39 ohm cm. Myxicola giant axons had an average resistivity of 68 ohm cm whereas that for barnacle giant muscle fibers was 178 ohm cm and from Amphiuma red blood cells was 350 ohm cm. The temperature dependence of conductivities of Aplysia neurons and squid axon was not different from that of equally conductive salt solutions. The cause of the extraordinarily high resistivities of some of these tissues is unknown, but the results suggest some mechanism whereby interactions of ions with cell water and/or macromolecules lower ionic mobility.

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